

## PCOS

# Relationship among plasma adipokines, insulin and androgens level as well as biochemical glycemic and lipidemic markers with incidence of PCOS in women with normal BMI

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Polycystic ovary syndrome (PCOS) is an endocrine disorder in women. Omentin-1 and vaspin are secretory adipokines that are produced by the visceral adipose tissue. These levels change in obese women with PCOS. The aim of this study is to investigate whether omentin and vaspin levels change in nonobese PCOS subjects. This study is a cross-sectional case control study in which 39 women with PCOS were picked out for this study. The inclusion criteria were based on the Rotterdam 2003 diagnostic criteria. The control group consisted of 39 women with normal pelvic sonographic reports having regular menstruation and showing no signs of infertility. The fasting plasma glucose (FPG), triglyceride (TG), Chol, and high-density lipoprotein cholesterol (HDL-C), insulin, testosterone, omentin and vaspin were measured by the enzymatic methods. The differences within these groups were calculated by the un-paired t-test and the Mann–Whitney test. The results from this study show a significant increase in the amount of insulin, testosterone, homeostasis model assessments for insulin resistance, TG and lower HDL in the patient group. No significant differences were seen in omentin, vaspin, FPG, Cho, low-density lipoprotein, very low-density lipoprotein cholesterol, blood urea nitrogen, Cr and homeostasis model assessments for B cell function levels between groups. Results show that PCOS is not a determinant of decreased omentin and vaspin plasma levels and those high androgen level and insulin resistances are warning signs of PCOS.

**Keywords:** Homeostasis model assessment, insulin resistance, omentin-1, polycystic ovary syndrome, vaspin

## Introduction

Polycystic ovary syndrome (PCOS) is the most commonly encountered endocrine disorder in women, affecting 5–10% of all women in the reproductive age [1]. PCOS is characterized by menstrual dysfunction and hyperandrogenism. It is associated with insulin resistance, pancreatic  $\beta$ -cell dysfunction, impaired glucose tolerance, type 2 diabetes, dyslipidemia and visceral obesity [2].

This metabolic syndrome (PCOS) is associated with an excessive accumulation of central body fat. The adipose tissue plays a role in energy storage and also it produces several hormones

and cytokines termed adipokines that have important role in the pathogenesis of insulin resistance, diabetes and atherosclerosis [3].

Omentin is a newly identified secretory adipokine that expressed by the visceral adipose tissue relative to subcutaneous adipose level [4–6]. *In vitro* studies have shown that omentin increase insulin signal transduction by activating the protein kinase Akt/protein kinase B and by enhancing insulin-stimulated glucose transport in isolated human adipocytes [4]. Most studies published so far have shown an inverse correlation of omentin with obesity [7,8]. Tan et al. [7] have reported that omentin is decreased in patients with PCOS, the disease which is commonly associated with insulin resistance and obesity.

Vaspin, another insulin-sensitizing adipokine derived from the visceral adipose tissue, plays an important role by inhibiting serine phosphorilase among insulin receptor-1 (irs-1) and insulin receptor-2 (irs-2) [9,10].

In some published articles, a negative correlation between obese PCOS with omentin and a positive association with vaspin plasma concentration was found [7,11,12]. Mahde et al. (2009) confirmed that these adipokines may be used for future diagnosis of PCOS with obesity.

So far, only one study has published about the effect of PCOS on visceral hormone derived; omentin in nonobese PCOS patients [13]. We conducted this study to investigate that omentin and vaspin change in nonobese PCOS patients as well as obese PCOS patients. In addition, we examined the plasma concentration of lipidemic and glycemic parameters and the insulin and the androgen hormones.

## Material and method

This study is a cross-sectional case control study. Seventy-eight women were studied in two equal member groups (the treatment group and the control group). Thirty-nine women  $21.68 \pm 4.01$  years old with PCOS who had been referred to the gynecological and obstetrics Abolfazl Specialized Clinic were chosen for this study. Inclusion criteria were based on the Rotterdam 2003 diagnostic criteria. The affected woman had to fulfill at least two criteria out of three (little or no ovulation, clinical hyperandrogenism and the showing up of a polycystic ovary in the sonogram) [14]. The control group consisted of

39 women in  $24.06 \pm 5.07$  year old range with a normal pelvis sonography, regular menstruation, no hysterectomy, no acne and no sign of infertility. Both groups matched in body mass index (BMI) and age.

They were excluded if they had a recent history (within 6 months) of myocardial infarction or stroke, significant liver or renal disease (plasma creatinine  $>130 \mu\text{mol/l}$ ), microproteinuria, thyroid disorders, neoplasm, diabetes, cushing, hyperprolactinemia and hypertension. They should not have taken contraceptive, glyocorticoid, ovulation stimulation, antihypertensive, oral hypoglycemic, weight loses, estrogen or antiandrogen medications. All patients fulfilled the following criteria: fasting plasma glucose (FPG)  $<110 \text{ mg/dl}$ , BMI  $<25 \text{ kg/m}^2$ , serum triglycerides (TGs)  $<150 \text{ mg/dl}$  and age  $<40$  years old. The study was approved by the Ethics Committee of the Medical Sciences of Bushehr University and the reported investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Also the participants in the project gave written consent.

Venous blood samples were taken between 8 a.m. and 9 a.m., in the follicular phase, between the first and the fourth day of menstruation. Plasma was obtained from the blood samples by adding 1 mg/ml Na<sub>2</sub>-EDTA. The blood samples were centrifuged at 3000 g for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at  $-80^\circ\text{C}$  for a period of no more than 6 weeks.

All measurements were carried out at the research laboratory of the Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Science. FPG was measured by the glucose-oxidase method of Pars Azmoon-co, Iran (intraassay and interassay coefficients of variation (CVs) were 3.5% and 3.0%, respectively). The total high-density lipoprotein cholesterol (HDL-C) levels (after precipitation with magnesium chloride) were measured by enzymatic techniques (Pars Azmoon-co, Iran); intraassay and interassay CVs were 1.5% and 1.9%, respectively. The low-density lipoprotein cholesterol (LDL-C) level was calculated by using the Friedewald formula [15].

The plasma insulin (intraassay and interassay CVs 2.8% and 3.5%, respectively), testosterone (intraassay and interassay CVs 3.1% and 4%, respectively), vaspin (intraassay and interassay CVs 3.8% and 4.5%, respectively) and omentin concentration (intraassay and interassay CVs 3.5% and 4.2%, respectively) were measured by enzyme-linked immunosorbant assay technique. (The insulin kit is the brand of DRG German Company and omentin-1 kit is the brand of APOTECHE Company). The insulin resistance was estimated by homeostasis model assessments for insulin resistance (HOMA-IR) and  $\beta$ -cell dysfunction calculated by homeostasis model assessments for B cell function (HOMA-B).

HOMA-IR was obtained by [16]:

$$\text{HOMA-IR} = \frac{\text{Insulin} \left( \frac{\mu\text{IU}}{\text{ml}} \right) \times \text{FBS} \left( \frac{\text{mmol}}{\text{ml}} \right)}{22.5}$$

HOMA-B was calculated by [17]:

$$\text{HOMA-B} = \frac{20 \times \text{Insulin} \left( \frac{\mu\text{IU}}{\text{ml}} \right)}{\text{FBS} \left( \frac{\text{mmol}}{\text{ml}} \right) - 3.5}$$

Weight and height was measured by using stadiometer standard techniques. The BMI was calculated by  $\text{kg weight/m}^2$  height.

## Statistical analysis

Statistical analysis of the data was performed using SPSS statistical software version 11.0. All results were expressed as mean  $\pm$  SD. The single sample Kolmogorov-Smirnov test was used to estimate the variables' distribution characteristics. Unpaired t-test was used to compare variables with normal distribution and Mann-Whitney for data with nonparametric distribution. With a calculated sample size of 87 persons for the PCOS and for the control groups, the study had the power of 90.5% to yield a statistically significant result between these two groups. Two-tailed probability value of  $p < 0.05$  was considered as statistically significant.

## Results

The distribution was not normal for omentin-1, vaspin and insulin and normal for FPG, TG, Chol, LDL-C, HDL-C, very low-density lipoprotein cholesterol (VLDL), blood urea nitrogen (BUN), Cr, testosterone, HOMA-B and HOMA-IR. Seventy-eight assigned subjects completed the study. The characteristics of the patients confirmed and the groups were well matched for all entry criteria (Table I). The result from this study shows that there is no significant variation in the amount of omentin, vaspin, plasma FPG, Cho, LDL, VLDL, BUN, Cr and HOMA-B in the case and the control groups. There is a significant increase in amount of insulin ( $p = 0.007$ ), testosterone ( $p = 0.009$ ), HOMA-IR ( $p = 0.005$ ), TG ( $p = 0.04$ ) and lower HDL ( $p = 0.014$ ) in the patient group as compared to the control group (Table II).

Table I. Baseline demographic characteristics of the patients ( $n = 78$ ). Means  $\pm$  SD.

Characteristic No.	Control group ( $n = 39$ )	Patient group ( $n = 39$ )	$p$ value
Age (years)	$24.06 \pm 6.58$	$21.68 \pm 4.01$	NS
Sex (n)			
Women	39	39	
Weight (kg)	$57.27 \pm 6.58$	$55.7 \pm 5.59$	NS
Height (cm)	$161.67 \pm 5.6$	$157.14 \pm 6.08$	NS
BMI ( $\text{kg/m}^2$ )	$21.87 \pm 1.83$	$22.58 \pm 2.14$	NS
WHR	$0.81 \pm 0.08$	$0.80 \pm 0.15$	NS

BMI, body mass index; WHR, waist-hip ratio. NS, Not significant.

Table II. Mean (SD) concentration in variables in polycystic ovary syndrome and control groups.

Characteristic	Control group	Patient group	$p$ value
Omentin-1 (ng/ml)	$26.89 \pm 18.69$	$22.47 \pm 13.31$	NS
Vaspin (ng/ml)	$1.13 \pm 1.9$	$0.90 \pm 1.4$	NS
Insulin ( $\mu\text{IU/ml}$ )	$13.53 \pm 5.20$	$17.91 \pm 7.41$	0.007
Testosterone (ng/ml)	$0.38 \pm 0.13$	$0.48 \pm 0.16$	0.009
Triglyceride (mg/dl)	$78.73 \pm 27.13$	$93.5 \pm 35.24$	0.04
Cholesterol (mg/dl)	$155.82 \pm 270.14$	$153.11 \pm 26.51$	NS
HDL-C (mg/dl)	$46.64 \pm 8.62$	$41.07 \pm 9.58$	0.014
LDL-C (mg/dl)	$92.41 \pm 21.19$	$93.23 \pm 23.45$	NS
VLDL-C (mg/dl)	$15.71 \pm 5.42$	$18.7 \pm 7.04$	NS
FPG (mg/dl)	$94.85 \pm 7.77$	$97 \pm 8.72$	NS
HOMA-IR	$3.05 \pm 1.06$	$3.84 \pm 1.08$	0.005
HOMA-B	$161.55 \pm 74.69$	$201.32 \pm 101.97$	NS
BUN (mg/dl)	$11.52 \pm 3.37$	$11.64 \pm 2.69$	NS
Creatinine (mg/dl)	$0.75 \pm 0.14$	$0.7 \pm 0.16$	NS

FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; BUN, blood urea nitrogen; HOMA-IR, homeostasis model assessments for insulin resistance; HOMA-B, homeostasis model assessments for B cell function. NS, Not significant. Data is presented as mean  $\pm$  SD.

## Discussion

Our data indicate that there is no significant differences in omentin and vaspin plasma level in PCOS with normal BMI compare to non-PCOS patients. Tan et al. [7] reported patients with PCOS I had lower omentin levels. The data of Muhed et al. (2009) showed a significant decrease in plasma omental adipokines levels in women with PCOS compared to that of the control group. The differences in BMI between obese PCOS patients in Muhed research and nonobese patients in our study can explain this discrepancy [11]. According to Karshow et al. (2004) adipose tissue is a complex essential and highly active metabolic and endocrine organ. Adipose tissue expresses and secretes cytokines such as adiponectin, resistin, omentin etc [3].

There is a controversy between our results, where no significant changes of vaspin plasma levels in nonobese PCOS women was observed and other studies which showed increased vaspin plasma levels in PCOS women [12,18,19]. In Cakal et al. and Tan et al. studies obese (BMI >27) PCOS women were presented with increased vaspin levels, while in Koiou et al. study both obese and nonobese PCOS women had elevated vaspin levels compared to matched controls. Increased vaspin levels in Koiou et al. [18] study might represent a compensatory mechanism to preserve insulin sensitivity and glucose tolerance which are impaired in obesity.

Our findings indicate a significant increase in HOMA-IR (insulin resistance) in PCOS subjects although our data didn't show any significant differences in FPG in the PCOS group. It can be concluded that as long as hyperinsulinemia is overcome by insulin resistance, glucose levels remain normal. We hypothesize that the high level of insulin in nonobese PCOS patients is responsible for the hyperandrogenism. It is possible that hyperinsulinemia as a result of insulin resistance causes the Luteinizing hormone (LH) effect on ovarian theca cells leading to androgen excess because the theca cells from PCOS women are intrinsically programmed to overproduce androgen [20]. Our data showed a significant higher concentration of testosterone in PCOS group as compared to the control group.

Studies with the euglycemic clamp technique indicate that insulin resistance is present in both obese and nonobese women with PCOS compared to normal women with the matched age and weight [21]. Dunaif et al. (1998) in accordance with our data have reported that insulin resistance is a unique and common finding in women with PCOS independent of obesity.

In this case control study, significant differences were seen between the PCOS group and the control groups in TG and HDL-C plasma concentration. Two novel studies in accordance with our findings showed a significant increase in TG and a significant decrease in HDL-c in PCOS patients [7,11]. The data from this small study suggest that dyslipidemia is secondary to excess androgen action in concert with the hyperinsulinemia associated with insulin resistance. Some studies had shown that treatment with nonsteroidal antiandrogen or insulin-sensitizing medication reduces circulating levels of TGs, LDL-C and the atherogenicity of the lipid profile in women with PCOS [22–25].

## Conclusion

If hyperandrogenism and insulin resistance are early warning signs of increasing risk of PCOS, these patients are prime candidates for preventive medicine. It is important that primary care providers begin to recognize these androgen and insulin parameters as a clue to screening of a complex, lifelong pattern,

potentially placing women at risk for PCOS but adipokines changes, including vaspin and omentin are consequences of fat cell accumulation but not PCOS in obese PCOS.

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